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Collagen fibre arrangement in the skin of the pig

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INTRODUCTION

In mammalian skin, collagen is the most abundant structural constituent of the dermis, comprising about three quarters of the dry weight of this part of the integument (Mathews, 1975). The ability of skin to resist the mechanical stresses of tension and pressure results from the arrangement and tensile strength of the fibres and fibre bundles formed by this scleroprotein. From the morphological point of view, the limited elasticity and extensibility, the constant tension and the shock absorption capacity of the skin depend on the three dimensional network of the collagen fibres and fibre bundles. This architecture has been investigated extensively in human skin, and has led to the description of several functional models of the mechanical characteristics of the dermis. The model concepts mostly rely on the two dimensional construction principles of a scissors lattice or a closely interwoven wire mesh, and are still under review (for literature see Gibson, Kenedi & Craik, 1965; Gibson & Kenedi, 1970; Szirmai, 1970; Brown, 1972; Hall, 1976; Meigel, Gay & Weber, 1977; Pfaller, Schuler, Schmidt & Dworschak, 1979).

The knowledge of collagen fibres and their architecture in the common integument of the pig is still fragmentary. Since it is possible that porcine skin may provide an experimental model for research into human skin (Meyer, Schwarz & Neurand, 1978*a*), it is reasonable to study the arrangement and proportion of these fibres in the dermis by applying different methods and comparing the skin of the wild boar, the domestic pig, and the miniature pig. The results obtained are discussed in the light of the various theories, mentioned above, concerning collagen fibre arrangement in the human dermis.

MATERIALS AND METHODS

Skin samples were obtained from the back, lateral body wall and abdomen of the following animals: 3 European wild boars (*Sus scrofa scrofa* L.) – 3 females (50–70 kg); 4 domestic pigs (German landrace) – 3 females (50–60 kg) and 1 castrated male (45 kg); 2 Hanford and 2 Göttingen miniature pigs – 3 females, 1 castrated male (30–40 kg).

For light microscopical examination, 10 μm fresh frozen horizontal and sagittal sections were used, which had been cut in a Slee cryostat (Type 'HS') and post-fixed with 4 % formalin–calcium (Lillie, 1965) for 15 minutes. Nomarski's interference contrast method was applied to sections which, without dehydration, had been embedded in glycerin–gelatine. Polarisation microscopy was applied to dehydrated sections which were embedded in a synthetic resin (Eukitt, Corbit–Balsam). The sections were viewed in Zeiss photomicroscopes I and II.

For examination by scanning electron microscopy, skin samples were fixed with 4 % formalin–calcium (Lillie, 1965) for 2–3 hours. 50–100 μm frozen sections were cut in the Slee cryostat, treated with hyaluronidase (from bovine testis, Sigma) in 0·1 M-phosphate buffer (pH 5·4) for 5 hours at 37 °C (Pfaller *et al.* 1979) and afterwards incubated with bacterial crude α-amylase (from *Bacillus subtilis*, Sigma) in 0·2 M-phosphate buffer (pH 5·4) for 90 hours at room temperature (see Finlay & Hunter, 1971). Sections were then washed with phosphate buffer and dehydrated through increasing concentrations of acetone, or directly transferred to isoamyl acetate. Finally, the specimens were mounted on copper stubs using silver paint (Emetron), coated with a thin layer of gold–palladium in a sputter coater (Balzers BAE 120, FN 102) and viewed in a JEOL JSM-35L scanning electron microscope operated at 25 kV.

RESULTS

Light microscopy

The use of undehydrated cryosections combined with Nomarski's interference contrast method showed that collagen fibres were arranged in broad bundles running in two main directions; in addition, several smaller fibre bundles and even single fibres were visible passing through the whole network in various directions. This method also clearly demonstrated that fibres branched off from one bundle and merged in a branch of the same bundle or in that of another bundle (Fig. 1).

Polarization microscopy of dehydrated cryosections generally showed the same arrangement of collagen fibres and fibre bundles, i.e. they were mainly orientated in two directions. This method was superior in demonstrating differences in fibre density and distribution within the dermis of the wild and domestic pigs used. A survey of these sections showed that collagen fibres were relatively sparse in the papillary layer, but abundant throughout the mid and the deep dermis (Figs. 2, 3). They were mostly arranged in bundles which, in the skin of the back and the lateral body wall, formed a dense lattice pattern (Fig. 3). In the subcutis, this texture became wider, a tendency which was even more evident in the subfascial fat layer (see also Meyer, Schwarz & Neurand, 1978*b*). In general, no clear beginning or ending of fibres or fibre bundles could be identified.

In all animals investigated, the number of collagen fibre bundles in skin from the abdominal region was smaller and a distinct lattice pattern of fibre arrangement was absent, not only in the papillary layer but also in the mid zone of the dermis. In contrast to the other body regions, the collagen fibres and fibre bundles here formed a relatively wide meshed pattern, partly with broader bundles running parallel to the epidermis, especially in the miniature pig (Figs. 4, 5).

When the tissues from different animals used were compared, various differences in collagen fibre bundle arrangement and content became obvious. In typical skin samples from the back and the lateral body wall of the wild boar, collagen fibre bundles were often relatively thick and long and penetrated into the subcutis (Fig. 6). In the domestic and miniature pig, this type of bundle arrangement was less pronounced. In contrast to the wild form, the domestic breeds exhibited a more compact layer of collagen fibres in the dermis of the back and the lateral body wall (Figs. 2, 3).

Scanning electron microscopy

The application of scanning electron microscopy confirmed that fibre bundles in the reticular layer of the dermis, in general, crossed each other in two main directions

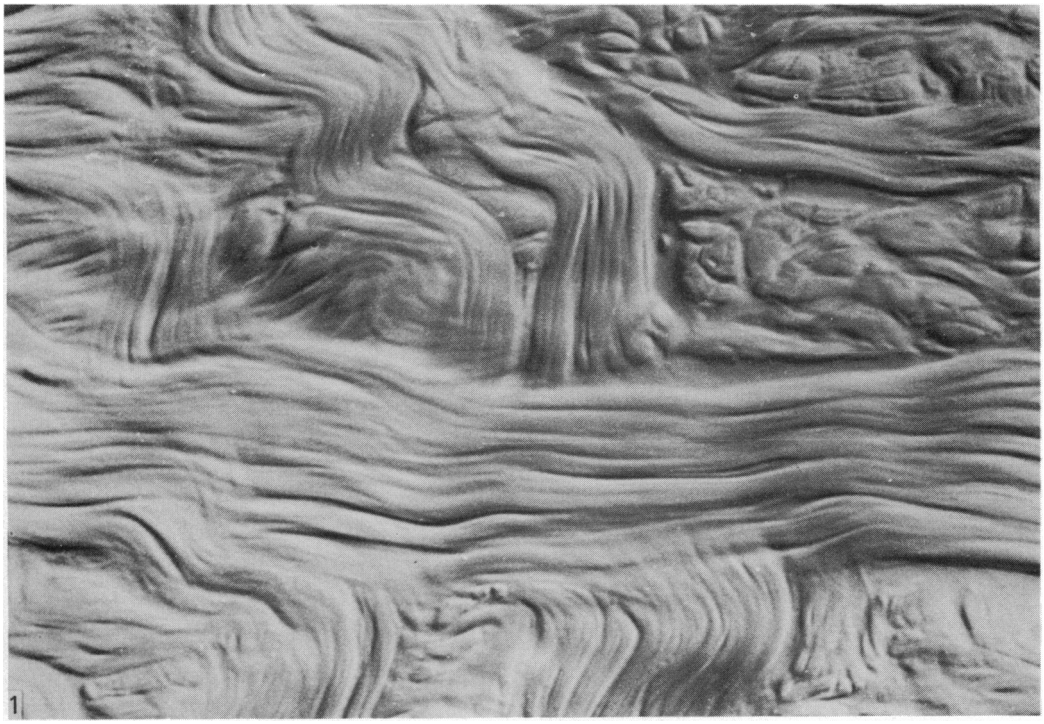
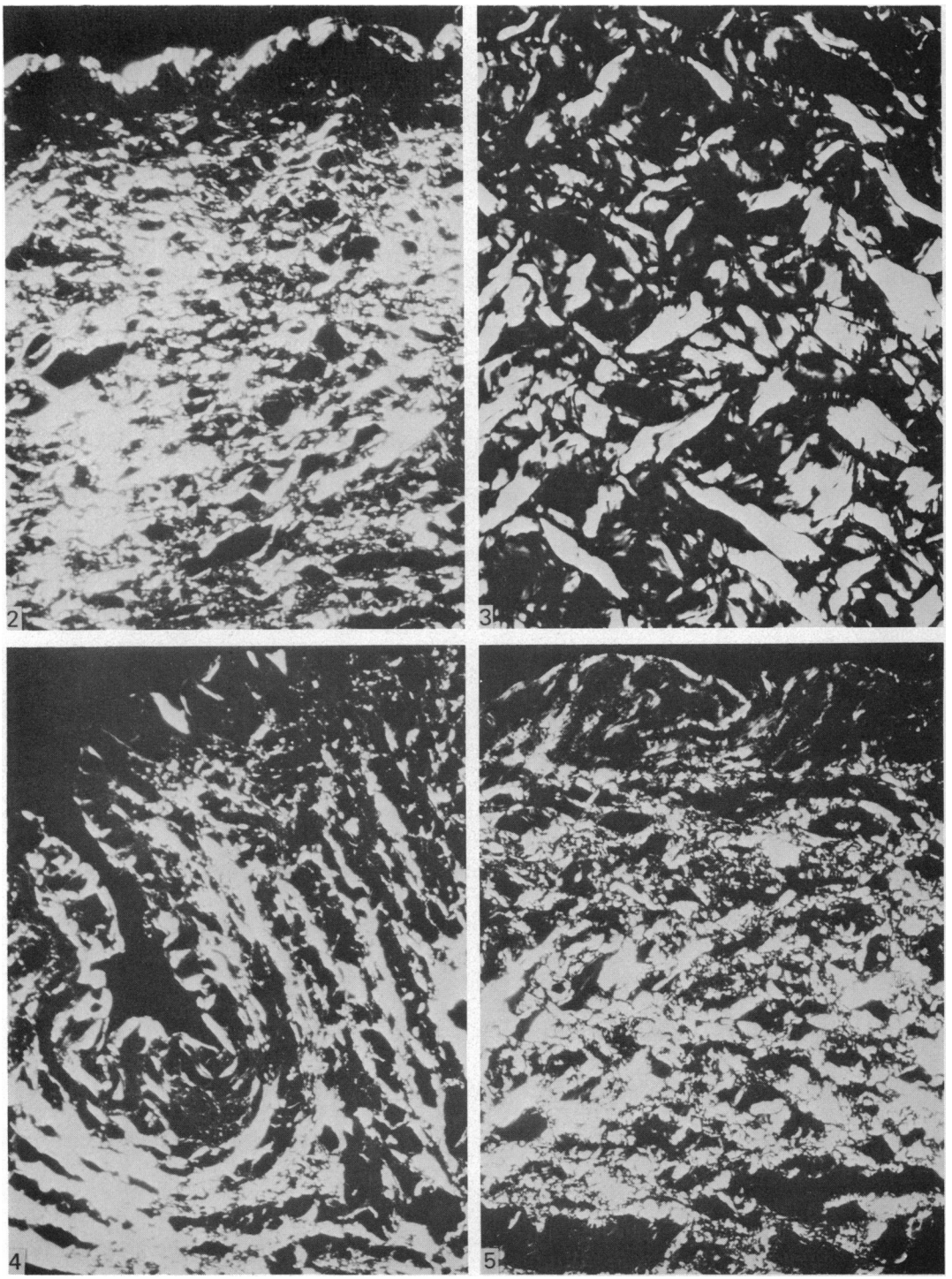


Fig. 1. Domestic pig, back; unstained sagittal cryosection and Nomarski's interference contrast method; crossing and branching of collagen fibre bundles are shown. × 1100.

(Figs. 7, 8), and that single fibres, or smaller fibre bundles, often emerged from one bundle and passed into another one, in this way closely interweaving the dense network. In addition, it was evident that a thin layer of elastic fibres was situated between the collagen fibre bundles. This method also showed differences in thickness and arrangement of fibres and fibre bundles between the papillary layer and the reticular layer of the pig skin. A network of finer and more horizontally orientated fibres and fibre bundles was confined to the relatively flat papillary zone of the dermis (Fig. 9). Finer fibres were also found around the pilosebaceous units (Fig. 10), the apocrine glands, and the blood vessels. When the dermis was horizontally sectioned, only a relatively small number of horizontally orientated and thicker collagen fibres or fibre bundles was detectable in the mid and deep zones (Fig. 11).

Larger and longer fibre bundles were visible, especially in the reticular layer of the wild boar. The domestic pig and the miniature pig exhibited comparatively densely packed fibre bundles, so that the dermis appeared to be massive and compact (Fig. 8).

The diameter of the collagen fibrils which could be identified at higher magnifications was about 0·06–0·1 μm, and these fibrils additionally showed a characteristic cross banding, as demonstrated in investigations of human dermal collagen (Finlay & Hunter, 1971).



Figs. 2–5. Polarisation microscopy. (2) Miniature pig, back. ×30. (3) Domestic pig, back. ×153. (4) Miniature pig, abdomen. ×17. (5) Domestic pig, abdomen. ×45. All are unstained and dehydrated sagittal cryosections.

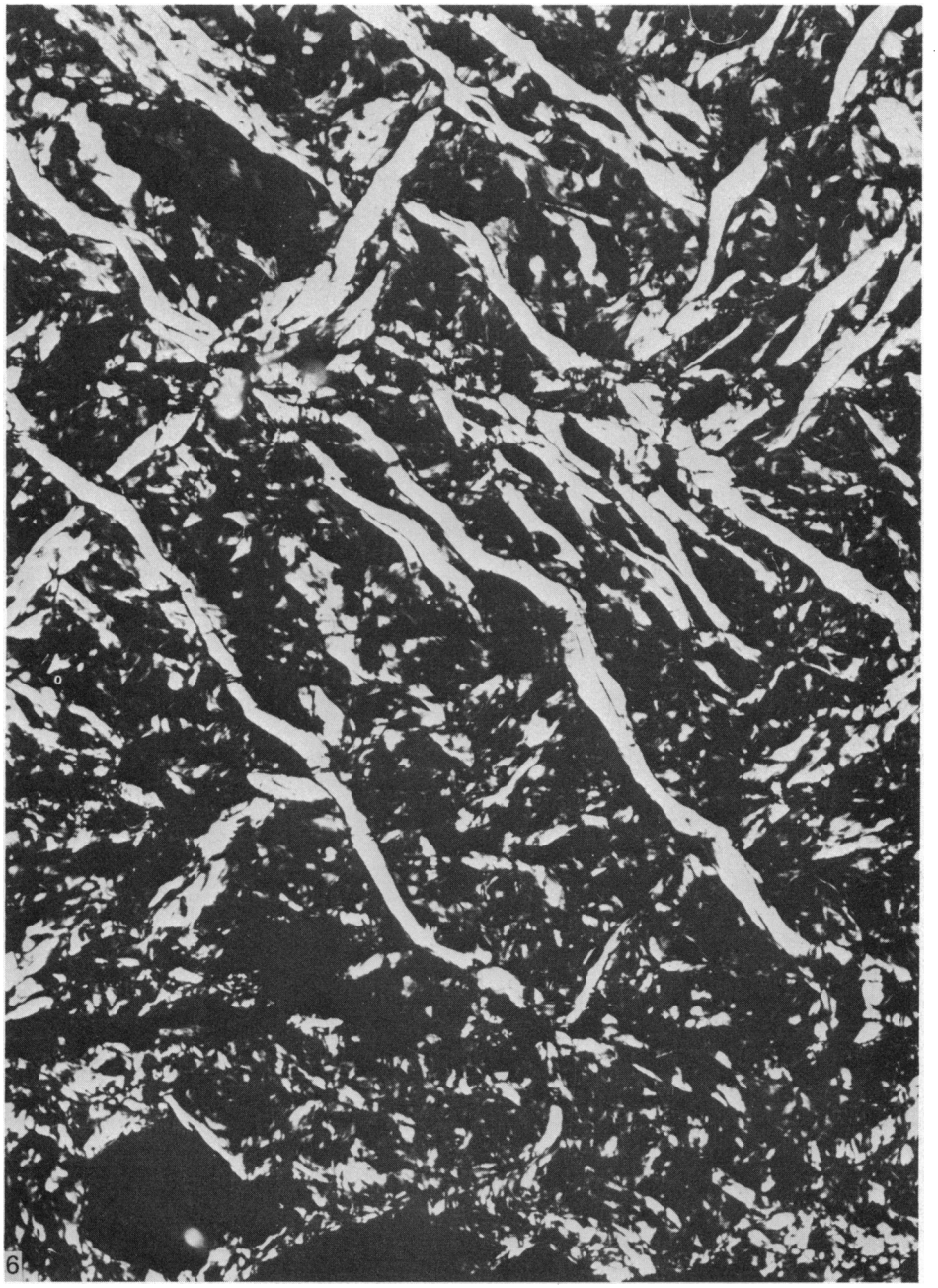
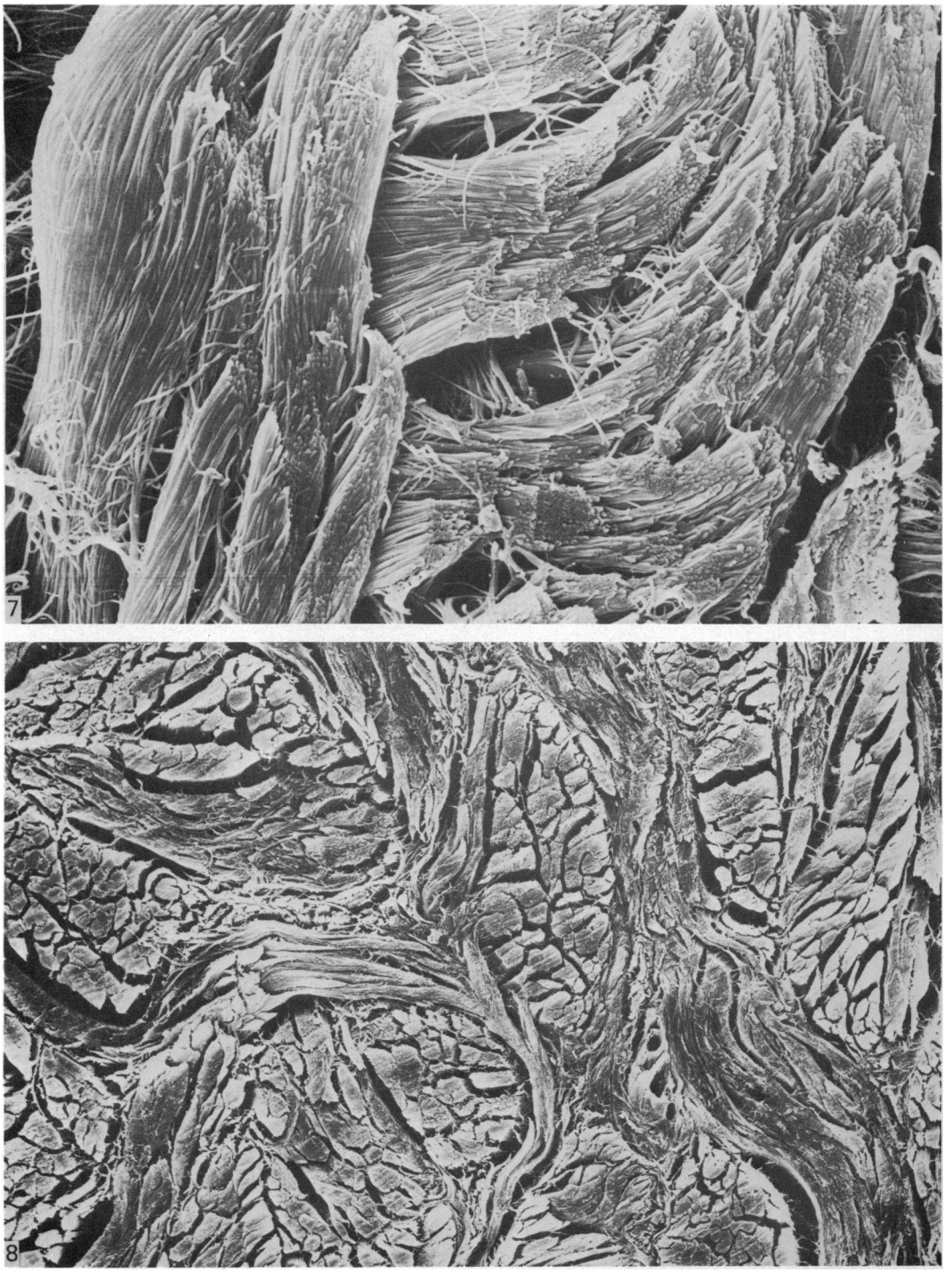


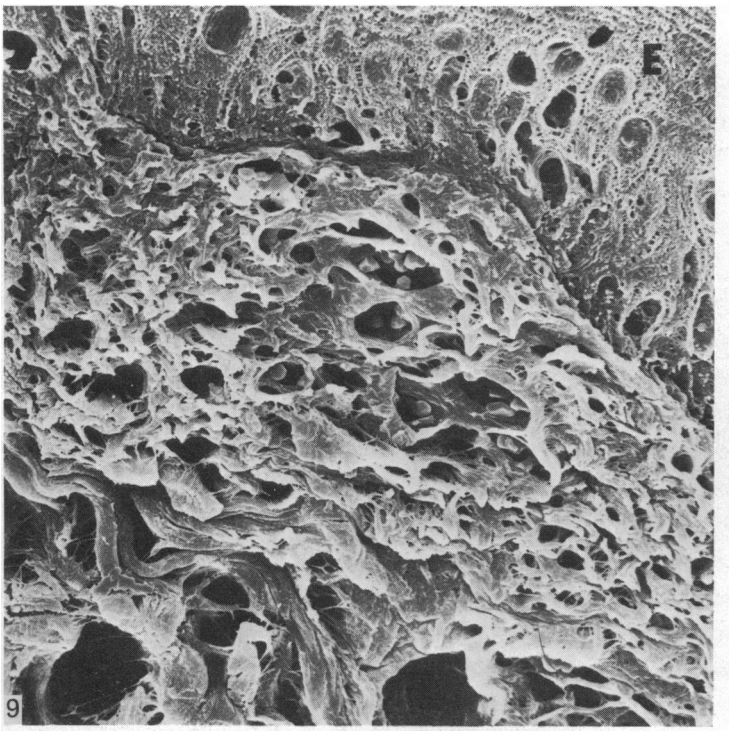
Fig. 6. Wild boar, mid and deep zones of dermis of back, by polarization microscopy; unstained and dehydrated sagittal cryosection. × 80.

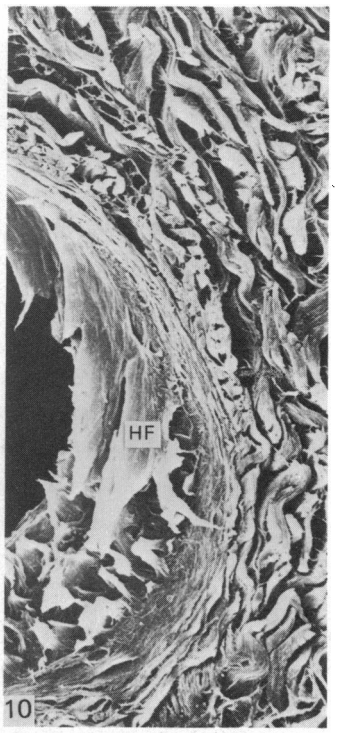
DISCUSSION

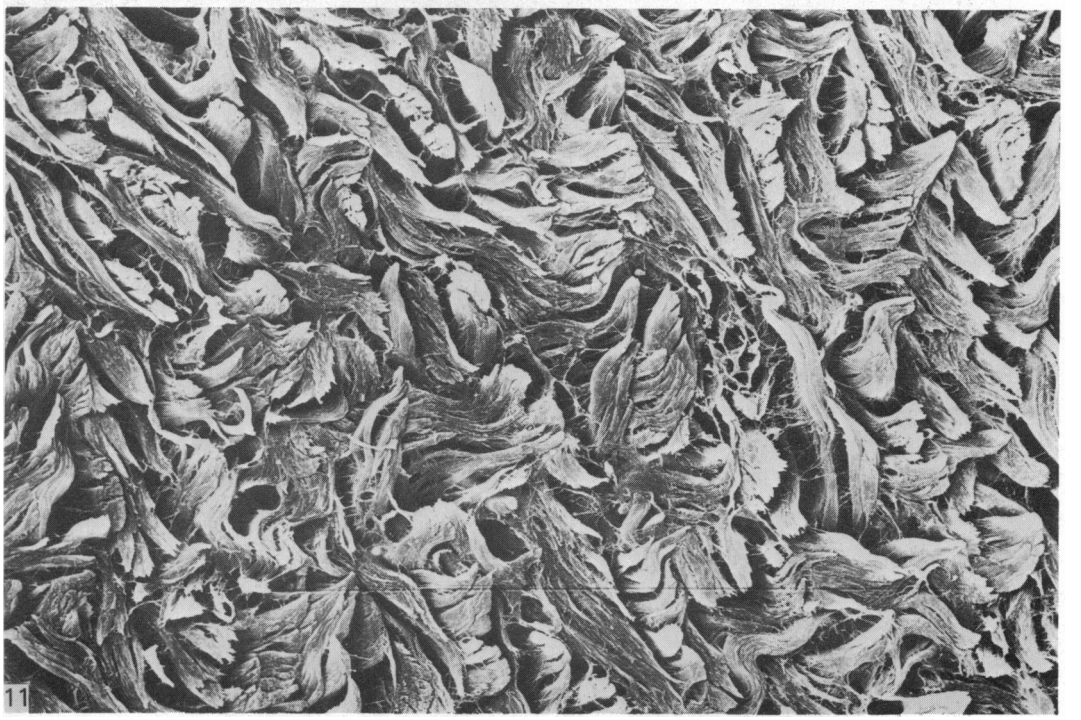
The results obtained during this investigation on the architecture of collagen fibres and fibre bundles in the dermis of the pig generally correspond to observations from human skin, including the thickness of collagen fibrils (domestic pig: 0·06–0·1 μm; man 0·07–0·1 μm) (for humans see Schmidt, 1968; Gibson & Kenedi, 1970; Szirmai,



Figs. 7, 8. Scanning electron microscopy. (7) Domestic pig, mid-dermis of lateral body wall. ×7550. (8) Miniature pig, mid-dermis of back. ×600; both are sagittal sections.







Figs 9–11. Scanning electron microscopy. (9) Domestic pig, papillary layer of dermis of back. (E, epidermis), sagittal section. ×1340. (10) Domestic pig, fibres around hair follicle in mid-dermis of back (HF). ×550. (11) Domestic pig, mid-dermis of back. ×720; 10 and 11 are horizontal sections.

1970; Finlay & Hunter, 1971; Pfaller *et al.* 1979; Elden, 1980). In summarising our different methods of visualising the arrangement of the collagen component of the dermis, it is obvious that a dense and massive three dimensional meshwork of fibres and fibre bundles dominates the bulk of this part of the skin of the pig, leaving little space for other tissue components. This is demonstrated best by the scanning electron microscope (see, e.g., Fig. 8).

In contrast to earlier investigations in porcine skin (Montagna & Yun, 1964), the orientation of the collagen fibres or fibre bundles was not found to be predominantly horizontal. The fibres and fibre bundles were mostly arranged in two main directions, i.e. they passed roughly obliquely between epidermis and subcutis. Single fibres and smaller fibre bundles also penetrated into the dermis in various other directions. All fibres and fibre bundles were interwoven by smaller fibres from one to another bundle.

Comparing the collagen fibre arrangement in the dermis of the wild boar with those in the domestic breeds, only the wild pig showed numerous large fibre bundles penetrating into the deep zone of the dermis and into the subcutis. The comparison of the different body regions clearly showed a relatively lower collagen content in the abdominal region of all animals investigated, especially in the papillary layer and the mid zone of the dermis. This is in agreement with the observation that this body region is normally characterized by a higher content of elastic fibres (Meyer, Neurand & Radke, 1981).

From the structural point of view, the mechanical properties of the dermis of the pig seem to depend largely on the rather rigid and stable network of its collagen component. This is especially true when considering that the longitudinal extensibility of collagen fibres and fibrils is not very great (Elden, 1980). On the other hand, the skin of the pig also comprises a relatively distinct elastic component, i.e. elastic fibres are found throughout the whole dermis forming a wide-meshed sponge (Meyer *et al.* 1981), which obviously causes the constant tension of the skin (for humans see Schmidt, 1968; Stüttgen & Schaefer, 1974; Elden, 1980). In summary, we have shown that a close interwoven pattern of elastic and collagen fibres exists in the skin of the pig. Such a structure may explain how elastic fibres are able to sustain a higher stress than would be predicted (see Ross, 1973). This may also be the reason for a relatively elastic solidity of the porcine skin. The decreased mobility of the skin of older pigs, however, may not be due to changes in dermal structure and function but to the immense deposition of fat in the subcutis.

The observations presented in this paper show that the domestic pig, more than the miniature pig, exhibits structural characteristics of collagen fibre arrangement in the dermis, which are comparable to human skin. In this connection, it may be of interest to note that collagen of porcine skin also shows similarities to that of human skin in the amino acid composition of the $\alpha 1$ and $\alpha 2$ chains (Heinrich *et al.* 1971). The structural as well as the biochemical properties of the collagen of porcine skin are important in view of the possible experimental value of the skin of the domestic pig for dermatological research (Meyer *et al.* 1978*a*). For example, wound healing and skin defects found in the domestic pig resemble those reported for man (for literature see Meyer & Neurand, 1976), and the observation that the collagen of porcine skin exhibits little or no antigenicity in human beings (Struck, 1971) accounts for its use both as a biological wound dressing and in transplantation surgery (for review see Kiene, Schill, Roewer & Frick, 1976).

SUMMARY

The arrangement and proportion of collagen fibres and fibre bundles in the dermis of the pig have been investigated with light microscopical (Nomarski's interference contrast, polarization optics) and scanning electron microscopical methods. Skin samples were obtained from different body regions of wild boars, domestic pigs and miniature pigs.

All the methods used have demonstrated that the bulk of the dermis is dominated by a massive three dimensional network of collagen fibres and fibre bundles, which cross each other in two main directions. Several smaller fibre bundles pass through the network in various other directions, constructing a densely interwoven fibre pattern. Differences were obvious between the body regions and the animals investigated.

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